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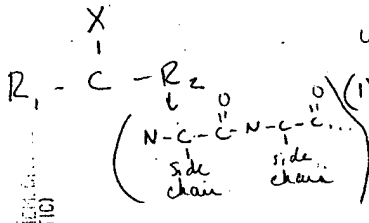
Search Topic:

Please write a detailed statement of search topic. Describe specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples or relevent citations, authors, keywords, etc., if known. For sequences, please attach a copy of the sequence. You may include a copy of the broadest and/or most relevent claim(s).

Please search the compounds:

X = Oxygen or Sulfur



wherein R_2 is a

(1) bombesin-like peptide or fragment thereof

↳ Specifically → gastrin-releasing (peptide or protein) GRP

Neuromedin B or C

and

(2) wherein R_1 is a fluorescent moiety

↳ ~~specif~~ specifically Bodipy, fluorescein, FITC or rhodamine.

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Point of Contact:
Susan Hanley
Technical Info. Specialist
CM1 12C14 Tel: 305-4053

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41

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Number of Databases:

Search Site	Vendors
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___ CM-1	___ STN
___ Pre-S	___ Dialog
Type of Search	___ APS
___ N.A. Sequence	___ Geninfo
___ A.A. Sequence	___ SDC
___ Structure	___ DARC/Questel
✓ Bibliographic	___ Other

PTO-1590 (9-90)

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     (FILE 'HOME' ENTERED AT 10:56:00 ON 16 JUL 2001)

     FILE 'HCAPLUS' ENTERED AT 10:56:09 ON 16 JUL 2001
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L1            32 S E4-9,E14-17
L2             1 S BONTER K?/AU
L3             0 S L1 AND L2
L4            33 S L1-2
L5        326968 S FLUORE?
L6             0 S L4 AND L5
L7             0 S L4 AND BOMBESIN?
L8            24 S L4 AND PEPTID?
L9          3477 S ?BOMBESIN?
L10       577675 S L5 OR FLUORO?
L11           93 S L9 AND L10
L12        34188 S FITC OR RHODAMINE OR FLUORESCEIN OR BODIPY
L13            6 S L11 AND L12
L14         1696 S GASTRIN-RELEASING
L15         2616 S L14 OR GRP
L16          831 S NEUROMEDIN

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                E BOMBESIN/CN
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                E GASTRIN RELEAS?CN
                E GASTRIN RELEAS?/CN
                E GASTRIN RELEASING/CN
L18            0 ES E4
                E NEUROMEDIN/CN
L19            2 S E3-4
                E NEUROMEDIN C/CN
L20            2 S E3
                E FLUORESCEIN/CN
L21            1 S E3
L22            1 S FITC/CN
                E RHODAMINE/CN
L23           58 S E4-96
L24            1 S BODIPY/CN

     FILE 'HCAPLUS' ENTERED AT 11:07:58 ON 16 JUL 2001
L25         3531 S L9 OR L17
L26        36914 S L12 OR L24 OR L23 OR L22 OR L21
L27          863 S L19-20 OR L16
L28           10 S (L25 OR L27 OR L15)(L)L26
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L37            4 S L35 AND (L10 OR L26)
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L39            7 S L38 NOT L28
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                E IMAGING AGENTS/CT
                E E3+ALL/CT
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L45           28 S L43 AND (?CONJUGAT? OR MODIFI? OR ATTACH? OR COVALENT? OR COU
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L47           26 S L46 NOT L44
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L49           87 S (L25 OR L27 OR L15)(S)(PEPTIDE(2A)BOND### OR AMIDE)
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no relevant hits from inventor search

L10 = fluorophores, etc in general
L26 = specifically named fluorophores
L25 = Bombesin
L27 = neuromedin
L15 = Gastric releasing prot or peptide

10 cites

using text terms

7 cites

using idexing

1 cite — broader - any prot

2 cites - type of bond w/ any protein

15 cites - any peptide/prot. conj. to fluorophore

L50 2 S L49(P)(L10 OR L26)
L51 7 S (L25 OR L27 OR L15)(P)(PEPTIDE(2A)BOND### OR AMIDE)(P)(L10 OR

7 cites

=> d bib abs l28 1

L28 ANSWER 1 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 2001:475803 HCAPLUS
TI Serine protease inhibitor causes F-actin redistribution and inhibition of calcium-mediated secretion in pancreatic acini
AU Singh, Vijay P.; Saluja, Ashok K.; Bhagat, Lakshmi; Hietaranta, Antti J.; Song, Albert; Mykoniatis, Andreas; Van Acker, Gijs J. D.; Steer, Michael L.
CS Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
SO Gastroenterology (2001), 120(7), 1818-1827
CODEN: GASTAB; ISSN: 0016-5085
PB W. B. Saunders Co.
DT Journal
LA English
AB The present study was undertaken to evaluate the role of serine proteases in regulating digestive enzyme secretion in pancreatic acinar cells. Isolated acini were stimulated by various secretagogues in the presence or absence of cell-permeant serine protease inhibitors 4-(2-aminoethyl)-benzenesulfonyl fluoride and N.alpha.-p-tosyl-L-phenylalanine chloromethyl ketone. F-actin distribution was studied after staining with **rhodamine** phalloidin. Both cell-permeant serine protease inhibitors blocked amylase secretion in response to secretagogues that use calcium as a second messenger (e.g., caerulein, carbamylcholine, and **bombesin**) but not to those that use adenosine 3',5'-cyclic monophosphate (cAMP) as a second messenger (e.g., secretin and vasoactive intestinal polypeptide). Incubation of the acini with these inhibitors also resulted in a dramatic redistribution of the F-actin cytoskeleton. This redistribution was energy dependent. Similar redistribution of F-actin from the apical to the basolateral region was also obsd. when acini were incubated with a supramaximally stimulating concn. of caerulein, which is known to inhibit secretion. These results suggest that a serine protease activity is essential for maintaining the normal apical F-actin distribution; its inhibition redistributes F-actin from the apical to the basolateral region and blocks secretion induced by secretagogues that act via calcium. CAMP reverses the F-actin redistribution and hence cAMP-mediated secretion is not affected.
RE.CNT 21
RE
(2) Andrews, B; Biochem Biophys Res Commun 2000, V273, P302 HCAPLUS
(4) Fruman, D; Methods 1996, V9, P146 HCAPLUS
(5) Harder, K; Biochem J 1994, V298, P395 HCAPLUS
(6) Kohli, V; Gastroenterology 1999, V116, P168 HCAPLUS
(7) Martin, B; J Biol Chem 1985, V260, P14932 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 128 2

L28 ANSWER 2 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:775653 HCAPLUS
DN 130:151143
TI Different putative neuromodulators are present in the nerves which distribute to the teleost skeletal muscle
AU Radaelli, G.; Domeneghini, C.; Arrighi, S.; Mascarello, F.; Veggetti, A.
CS Department of Animal Sciences, Faculty of Veterinary Medicine, University of Padova, Italy
SO Histol. Histopathol. (1998), 13(4), 939-947
CODEN: HIHIES; ISSN: 0213-3911
PB Histology and Histopathology
DT Journal
LA English
AB The presence of putative neuromodulators in the nerve fibers was investigated in white skeletal muscle of 2 teleost fish not taxonomically correlated and showing different patterns of innervation (multiple vs. focal innervation). Cryostat sections of epaxial, hypaxial, and adductor mandibulae (AM) muscles of Sparus aurata and Anguilla anguilla were stained histochem. for NADPH-diaphorase. Other sections were used for indirect immunohistochem. (streptavidin-biotin and **rhodamine** immunofluorescence methods), employing antibodies specific for putative excitatory or inhibitory peptides, including CGRP, substance P, Met-enkephalin, **bombesin**, and VIP. In addn., ultrastructural observations were performed to describe the morphol. of the motor endplates. A strong immunoreactivity for CGRP and substance P was found in many nerve terminals. Met-enkephalin, **bombesin**, and VIP immunoreactivities were less frequently obsd. No immunoreactivity was obsd. to cholecystokinin, neuropeptide Y, or 5-HT. NADPH-diaphorase was identified in nerve fibers of the AM complex only of A. anguilla. Electron microscopy observations evidenced >1 type of synaptic vesicle in motor endplates. Some differences in putative neuromodulator distributions were obsd. in the 2 species and muscle complexes, which may be related to the different taxonomical position as well as the different pattern of innervation of white muscle fibers.
RE.CNT 49
RE
(1) Andersen, A; Int Rev Cytol 1992, V138, P89 HCAPLUS
(3) Bell, T; Neurosci Lett 1997, V226, P187 HCAPLUS
(6) Conlon, J; J Neurochem 1991, V56, P1432 HCAPLUS
(7) Csillik, B; J Histochem Cytochem 1993, V41, P1547 HCAPLUS
(8) Dawson, T; Proc Natl Acad Sci USA 1991, V88, P7797 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs l28 3

L28 ANSWER 3 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:617841 HCAPLUS
DN 129:311098
TI A role for the p38 mitogen-activated protein kinase/Hsp 27 pathway in cholecystokinin-induced changes in the actin cytoskeleton in rat pancreatic acini
AU Schafer, Claus; Ross, Sarah E.; Bragado, M. Julia; Groblewski, Guy E.; Ernst, Stephen A.; Williams, John A.
CS Departments of Physiology and Internal Medicine, University of Michigan, Ann Arbor, MI, 48109-0622, USA
SO J. Biol. Chem. (1998), 273(37), 24173-24180
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB Cholecystokinin (CCK) and other pancreatic secretagogues have recently been shown to activate signaling kinase cascades in pancreatic acinar cells, leading to the activation of extracellular signal-regulated kinases and Jun N-terminal kinases. The authors now show the presence of a third kinase cascade activating p38 mitogen-activated protein (MAP) kinase in isolated rat pancreatic acini. CCK and osmotic stress induced by sorbitol activated p38 MAP kinase within minutes; their effects were dose-dependent, with maximal activation of 2.8- and 4.4-fold, resp. The effects of carbachol and **bombesin** on p38 MAP kinase activity were similar to those of CCK, whereas phorbol ester, epidermal growth factor, and vasoactive intestinal polypeptide stimulated p38 MAP kinase by 2-fold or less. Both CCK and sorbitol also increased the tyrosyl phosphorylation of p38 MAP kinase. Using the specific inhibitor of p38 MAP kinase, SB 203580, the authors found that p38 MAP kinase activity was required for MAP kinase-activated protein kinase-2 activation in pancreatic acini. SB 203580 reduced the level of basal phosphorylation and blocked the increased phosphorylation of Hsp 27 after stimulation with either CCK or sorbitol. CCK treatment induced an initial rapid decrease in total F-actin content of acini, followed by an increase after 40 min. Preincubation with SB 203580 significantly inhibited these changes in F-actin content. Staining of the actin cytoskeleton with **rhodamine**-conjugated phalloidin and anal. by confocal fluorescence microscopy showed disruption of the actin cytoskeleton after 10 and 40 min of CCK stimulation. Pretreatment with SB 203580 reduced these changes. These findings demonstrate that the activation of p38 MAP kinase is involved not only in response to stress, but also in physiol. signaling by gastrointestinal hormones such as CCK, where activation of Gq-coupled receptors stimulates a cascade in which p38 MAP kinase activates MAP kinase-activated protein kinase-2, resulting in Hsp 27 phosphorylation. Activation of p38 MAP kinase, most likely through phosphorylation of Hsp 27, plays a role in the organization of the actin cytoskeleton in pancreatic acini.

=> d bib abs l28 4

L28 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:633567 HCAPLUS
DN 127:303487
TI Bombesin-like peptides stimulate somatostatin release from rat fundic D cells in primary culture
AU Schaffer, Kirsten; Herrmuth, Hedda; Mueller, James; Coy, David H.; Wong, Helen C.; Walsh, John H.; Classen, Meinhard; Schusdziarra, Volker; Schepp, Wolfgang
CS Dep. Med. II and Pathology, Technical Univ., Munich, D-81675, Germany
SO Am. J. Physiol. (1997), 273(3, Pt. 1), G686-G695
CODEN: AJPHAP; ISSN: 0002-9513
PB American Physiological Society
DT Journal
LA English
AB In several species, **bombesin**-like neuropeptides stimulate somatostatin release in in vitro prepns. of gastric mucosa. We sought to det. if this response is due to a direct effect on fundic D cells. Rat fundic mucosal cells were isolated by pronase E (1% D cells). D cells were sepd. by counterflow elutriation and subsequent d.-gradient centrifugation (Nycodenz) (15% D cells) and grown in primary culture for 48 h (46% D cells). Cultured cells were double stained with affinity-purified rabbit-anti- **gastrin-releasing** peptide (**GRP**) receptor antibody and mouse monoclonal antibody to human somatostatin. After incubation with **rhodamine**-labeled anti-rabbit and **fluorescein** isothiocyanate-labeled anti-mouse antibodies, reactions were visualized by fluorescence microscopy. All cells pos. for somatostatin had **GRP** receptors, whereas all non-D cells showed no expression in this G cell-free culture system. Somatostatin release from cultured cells was stimulated by sulfated cholecystokinin octapeptide (CCK-8; EC50 3 x 10-10 M) and epinephrine (EC50 4 x 10-8 M), which are established stimuli for canine fundic D cells. **Bombesin** (EC50 6 x 10-11 M), its mammalian analog **GRP**-27, and **neuromedin** C (**GRP**-10)(EC50 1 x 10-10 M, for both) were almost equally potent stimuli of somatostatin release, eliciting maximal response at 10-9 M (400-550% above basal). **Neuromedin** B was less potent and effective (maximal response at 10-8 M, 230% above basal). [D-Phe6]**bombesin**-(6-13)-OMe, a specific **bombesin** receptor antagonist, inhibited **bombesin**-stimulated somatostatin release in a competitive manner (IC50 9 x 10-8 M). Potentiating interactions were obsd. between **bombesin** and dibutyryladenosine 3',5'-cyclic monophosphate (dbcAMP) or epinephrine, but not between **bombesin** and CCK-8. We conclude that **bombesin**-like peptides directly stimulate somatostatin release by interacting with specific receptors on rat fundic D cells. **Bombesin**-like peptides appear to induce Ca2+-phospholipid-dependent signal-response transduction, as is indirectly suggested by potentiating interactions with dbcAMP or epinephrine.

=> d bib abs l28 5

L28 ANSWER 5 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1996:381014 HCAPLUS
DN 125:51226
TI Pasteurella multocida toxin stimulates mitogenesis and cytoskeleton reorganization in Swiss 3T3 fibroblasts
AU Dudet, Laure I.; Chailler, Pierre; Dubreuil, J. Daniel; Martineau-Doize, Beatrice
CS Caculte Medecine Veterinaire, Univ. Montreal, St-Hyacinthe, PQ, J2S 7C6, Can.
SO J. Cell. Physiol. (1996), 168(1), 173-182
CODEN: JCLLAX; ISSN: 0021-9541
DT Journal
LA English
AB In the present study designed to further investigate the effects of Pasteurella multocida toxin (PMT) on cell shape and proliferation, we report that the mitogenic effect of affinity-purified PMT on quiescent 3T3 cells was even superior at 5 ng/mL to that of fetal bovine serum (FBS) or **bombesin**. This pos. effect was inhibited by heat denaturation and methylamine treatment (this agent blocks internalization). Preincubation of PMT with gangliosides GM1, GM2, or GM3 counteracted its effect on DNA synthesis, suggesting that the toxin binds to GM-type ceramides on target cells. The distribution of F-actin was analyzed in control/treated cells using **FITC**-conjugated phalloidin. In comparison with FBS and **bombesin**, PMT triggered a more rapid and profound reorganization of cortical actin into prominent stress fibers after only 5-10 min. This event led to the retraction of cells after only 30 min and ultimately to the induction of mitotic figures. Interestingly, methylamine blocked the effects of PMT on stress fiber formation and cell retraction but not the ruffling response, suggesting that some early events may not require toxin internalization. In summary, these findings indicate that PMT concomitantly exerts a strong mitogenic activity and a rapid stimulation of cytoskeletal rearrangements, possibly after binding to membrane gangliosides and subsequent internalization. We propose that this toxin could be used in the future as a defined inducer of transduction signals involved in cellular proliferation and control of cell shape.

=> d bib abs 128 6

L28 ANSWER 6 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1996:298266 HCAPLUS
DN 124:315130
TI Confocal laser scanning microscopy examination of cell distribution in macroporous microcarriers
AU Bancel, Stephane; Hu, Wei-Shou
CS Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN, 55455-0132, USA
SO Biotechnol. Prog. (1996), 12(3), 398-402
CODEN: BIPRET; ISSN: 8756-7938
DT Journal
LA English
AB Macroporous microcarriers are often used to cultivate animal cells. The pores in the interior of the beads provide surface and space for cell growth. It is not clear how anchorage-dependent and suspension cells populated these microcarriers during cultivation. Confocal laser scanning microscopy was employed to perform time lapse observation of the cells in the interior. The structure of the bead was stained with **fluorescein** isothiocyanate for visualization, while the cells were stained with dialkyl indocarbocyanines for tracking over time. It was obsd. that mouse fibroblastic cells CRE BaG2 did not move extensively after initial attachment. Some cell divisions were obsd. during the course of the expts., and essentially all cells remained viable throughout. Few hybridoma cells were deposited into the pores in the interior of the microcarriers. The results suggest that the occupancy of the internal vol. by cells after prolonged cultivation is largely due to the growth of cells that are deposited in the interior as opposed to the migration of cells from the external surface into the interior. This method of observing cell behavior in a three-dimensional structure may find applications in other three-dimensional cell culture systems. The animation of time lapse sections is available on the worldwide web at http://www.cems.umn.edu/.apprx.wshu_**grp**/acre/microcarrier.html.

=> d bib abs 128 7

L28 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1995:660294 HCAPLUS
DN 123:314467
TI Modification of (Lys3)-**bombesin** with **fluorescein** 5'-isothiocyanate
AU Alberto, Mazzini; Cristina, Ruggi; Roberta, Bedotti; Paolo, Cavatorta; Roberto, Favilla
CS Department of Physics, University of Parma, Parma, 43100, Italy
SO Med., Biol., Environ. (1993), 21(2), 619-28
CODEN: MBENDX; ISSN: 0302-0800
DT Journal
LA English
AB **Fluorescein** 5'-isothiocyanate (**FITC**) was used to modify the lysine residue of (Lys3)-**bombesin**, a fully active analog of the hormone **bombesin**. The incorporation of **FITC** was performed at alk. pH, where the stability of **FITC** and (Lys3)-**bombesin** were previously analyzed. The fluorescent peptide was purified chromatog. on a hydrophobic interaction column and spectroscopically characterized. Preliminary expts. on proliferation of cultured Swiss 3T3 cells indicated that **FITC** labeling does not alter the biol. activity of the modified hormone.

=> d bib abs 128 8

L28 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1995:410215 HCAPLUS
DN 122:178902
TI Direct observation of endocytosis of gastrin releasing peptide and its receptors
AU Grady, Eileen F.; Slice, Lee W.; Brant, William O.; Walsh, John H.; Payan, Donald G.; Bunnett, Nigel W.
CS Departments of Surgery, Physiology and Medicine, Univ. of California, San Francisco, CA, 94143, USA
SO J. Biol. Chem. (1995), 270(9), 4603-11
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Endocytosis of the **gastrin releasing** peptide receptor (**GRP**-R) may regulate cellular responses to **GRP**. The authors obsd. endocytosis in transfected epithelial cells by confocal microscopy using cyanine 3-**GRP** (cyanine 3.18-labeled **gastrin releasing** peptide) and **GRP**-R antibodies. At 4.degree., cy3-**GRP** and **GRP**-R were confined to the plasma membrane. After 5 min at 37.degree., ligand and receptor were internalized into early endosomes with **fluorescein** isothiocyanate-transferrin. After 10 min, cy3-**GRP** and **GRP**-R were in perinuclear vesicles, and at 60 min cy3-**GRP** was in large, central vesicles, while **GRP**-R was at the cell surface. The authors quantified surface **GRP**-R using an antibody to an extracellular epitope and an 125I-labeled secondary antibody. After exposure to **GRP**, there was a loss and subsequent recovery of surface **GRP**-R. Recovery was unaffected by cycloheximide, and thus independent of new protein synthesis, but was attenuated by acidotropic agents, and therefore required endosomal acidification. Internalization of 125I-**GRP**, assessed using an acid wash, was maximal after 10-20 min, and was clathrin-mediated since it was inhibited by hyperosmolar sucrose and phenylarsine oxide. Thus, **GRP** and its receptor are rapidly internalized into early endosomes and then dissoc. in an acidified compartment. **GRP** is probably degraded whereas the **GRP**-R recycles.

=> d bib abs 128 9

L28 ANSWER 9 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1994:601751 HCAPLUS
DN 121:201751
TI Agonist-induced rise in intracellular calcium of lens epithelial cells: effects on the actin cytoskeleton
AU Rafferty, Nancy S.; Rafferty, Keen A.; Ito, Etsuro
CS Northwestern University, Chicago, IL, USA
SO Exp. Eye Res. (1994), 59(2), 191-201
CODEN: EXERA6; ISSN: 0014-4835
DT Journal
LA English
AB Primary cultures of rabbit and skate lens epithelia were used to investigate the effect of calcium release from intracellular stores upon the actin cytoskeleton. Primary cultures were loaded with fura-2 AM and intracellular calcium, i.e., (Ca2+)i, was quantitated using a Hamamatsu Photonics digital imaging system. Agonists used were **bombesin**, inositol-1,4,5-trisphosphate (IP3), thapsigargin (Tg), neuropeptide Y (NPY) and calcium chloride. Recordings were typically made on seven cells in each case. The authors found that IP3 caused a 6-fold immediate release of (Ca2+)i in rabbit cells, but skate cells showed no response unless permeabilized with saponin, whereupon an increase of about 50% occurred. Tg induced release from internal stores in rabbit cells, but had no effect on skate cells. **Bombesin** caused a large increase in (Ca2+)i release in both, while NPY had no effect in either. Skate cells incubated in calcium-free EGTA-Ringer's soln. responded rapidly to addn. of 5 mM CaCl2, whereas only three of 35 rabbit cells responded, and in gradual fashion. After calcium imaging, the cells were fixed and stained with **rhodamine** phalloidin or with an antibody against IP3 receptor (IP3R) conjugated to **FITC**. Fluorescence microscopy revealed that the actin cytoskeleton had reorganized from the normal stress fiber pattern into polygonal networks. Tg caused the same structures to form in rabbit cells, but **bombesin** had no effect. IP3 receptor was located intracellularly, presumably on endoplasmic reticulum, and was not assocd. with plasma membranes. The rapid response of rabbit cells may have been caused by the DMSO in which fura-2 was dissolved. The authors have found an interesting difference in agonist-induced calcium release between rabbit and skate cells. The latter may utilize either a Ca-Na exchanger or capacitative calcium entry, which could reflect a difference in lens accommodative mechanisms. This seems relevant in view of the fact that the rabbit lens accommodates through change in shape, whereas the skate lens does so through translation of position.

=> d bib abs l28 10

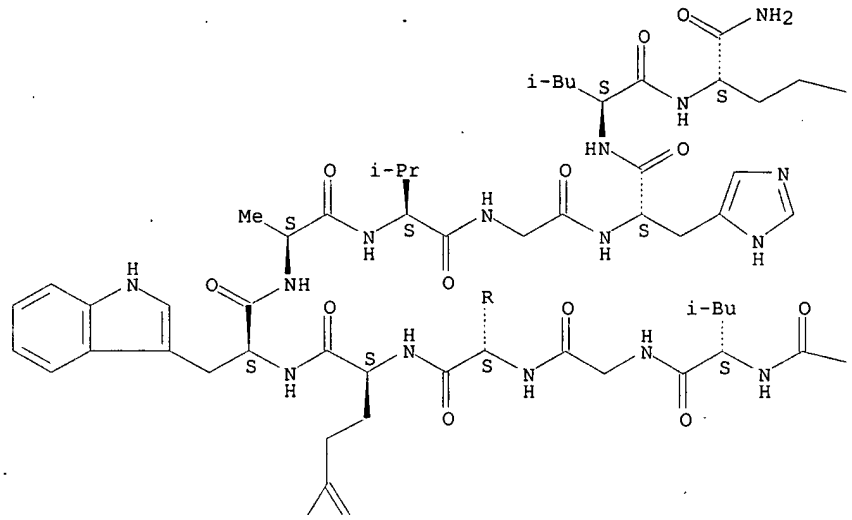
L28 ANSWER 10 OF 10 HCAPLUS COPYRIGHT 2001 ACS
AN 1991:401318 HCAPLUS
DN 115:1318
TI Biotinylation of a bombesin/gastrin-releasing peptide analog for use as a receptor probe
AU Anton, Peter A.; Reeve, Joseph R., Jr.; Rivier, Jean E.; Vidrich, Alda; Schepp, Wolfgang; Shanahan, Fergus
CS Dep. Med., UCLA, Los Angeles, CA, 90024, USA
SO Peptides (Fayetteville, N. Y.) (1991), 12(2), 375-81
CODEN: PPTDD5; ISSN: 0196-9781
DT Journal
LA English
AB The development of a biotinylated **bombesin-gastrin-releasing** peptide (**GRP**) for use as a receptor probe is reported. The lysine13 of a **GRP**-27 was substituted by arginine and lysine was added to the amino terminus. Biotinylation of the N-terminal lysine was performed. The biotinylated peptide was purified by HPLC and characterized by mass spectral anal. Binding studies with murine Swiss 3T3 fibroblasts, cells known to express **bombesin/GRP** receptors, yielded a dissocn. curve for the biotinylated **GRP**-27 analog (biotin-Lysyl[Asp12,Arg13]**GRP**-27) which was nearly identical to that of native **GRP**. Using studies of gastrin release from isolated canine G cells, equipotent functional activity of the biotinylated probe and unmodified **GRP** was demonstrated. Measurements of retained 125I-avidin confirmed that the biotin/avidin interaction could occur once the biotin-peptide complex was bound. Applicability of the probe was demonstrated with fluorescent microscopy using avidin-**FITC** on Swiss 3T3 fibroblasts. In conclusion, a novel biotinylated **bombesin/GRP** analog has been developed which retains the functional characteristics of the native peptide and is a useful probe for receptor studies.

=> d bib abs hitstr 1

L39 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:878948 HCAPLUS
DN 134:161051
TI G protein-coupled receptor signaling in human ductal pancreatic cancer cells: neurotensin responsiveness and mitogenic stimulation
AU Ryder, Nova M.; Guha, Sushovan; Hines, Oscar J.; Reber, Howard A.; Rozengurt, Enrique
CS Department of Surgery School of Medicine, University of California, Los Angeles, CA, 90095-1786, USA
SO J. Cell. Physiol. (2001), 186(1), 53-64
CODEN: JCLLAX; ISSN: 0021-9541
PB Wiley-Liss, Inc.
DT Journal
LA English
AB Neuropeptides and their corresponding G protein-coupled receptors (GPCRs) are increasingly implicated in the autocrine/paracrine stimulation of growth of human cancers. We report that neurotensin induced rapid Ca2+ mobilization from intracellular stores followed by Ca2+ influx in five human ductal pancreatic cancer cell lines: HPAF-II, Capan-1, Capan-2, PANC-1, and MIA PaCa-2. In addn., most cell lines exhibited Ca2+ responses to multiple neuropeptides including bombesin, bradykinin, cholecystokinin, and vasopressin and to bioactive lipids, including lysophosphatidic acid (LPA), that also act via GPCRs. The well-differentiated line HPAF-II responded to at least seven independent GPCR agonists. The concns. of neurotensin required to induce half-maximal effects (EC50) in HPAF-II and PANC-1 cells were 5 and 8nM, resp. Digital **fluorescence** image anal. to measure Ca2+ responses in single cells revealed that 90% or more of HPAF-II and PANC-1 cells responded to 10nM neurotensin. Addn. of neurotensin to PANC-1 cells also induced rapid and dose-dependent extracellular-regulated protein kinase (ERK-1 and ERK-2) activation and subsequently, stimulated DNA synthesis. The signaling complexity of GPCRs uncovered by these studies reveals a new aspect in the biol. of human pancreatic cancer and could offer the basis for new approaches to the treatment of this disease.
IT **31362-50-2, Bombesin**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(G protein-**coupled** receptor signaling in human ductal pancreatic cancer cells: neurotensin responsiveness and mitogenic stimulation)
RN 31362-50-2 HCAPLUS
CN Bombesin (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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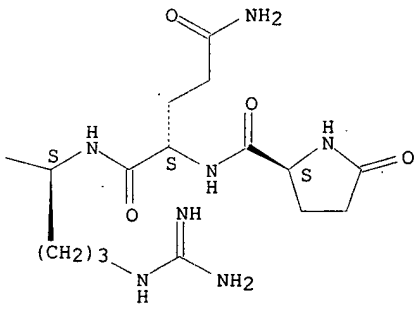

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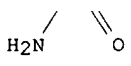
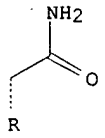




RE.CNT 47
RE
(1) Alessi, D; J Biol Chem 1995, V270, P27489 HCAPLUS
(2) Avis, I; Mol Carcinog 1993, V8, P214 HCAPLUS
(4) Castagliuolo, I; J Clin Invest 1999, V103, P843 HCAPLUS
(5) Detjen, K; Gastroenterology 1997, V112, P952 HCAPLUS
(6) Elek, J; Anticancer Res 2000, V20, P53 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 2

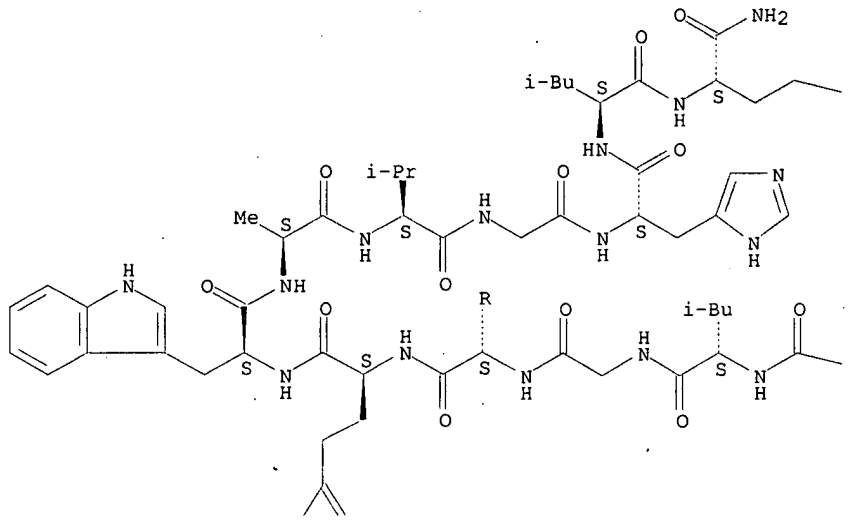
L39 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:842014 HCAPLUS
DN 134:21520
TI Novel cyanine and indocyanine dye bioconjugates for biomedical applications
IN Achilefu, Samuel; Dorshow, Richard Bradley; Bugaj, Joseph Edward; Rajagopalan, Raghavan
PA Mallinckrodt Inc., USA
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000071162	A2	20001130	WO 2000-US11060	20000426
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 6217848	B1	20010417	US 1999-325769	19990604
PRAI	US 1999-135060	P	19990520		
	US 1999-325769	A	19990604		

OS MARPAT 134:21520
AB Dye-peptide conjugates useful for diagnostic imaging and therapy are disclosed. The dye-peptide conjugates include several cyanine dyes with a variety of bis- and tetrakis(carboxylic acid) homologs. The small size of the compds. allows more favorable delivery to tumor cells as compared to larger mol. wt. imaging agents. The various dyes are useful over the range of 350-1300 nm, the exact range being dependent upon the particular dye. Use of dimethylsulfoxide helps to maintain the **fluorescence** of the compds. The mols. of the invention are useful for diagnostic imaging and therapy, in endoscopic applications for the detection of tumors and other abnormalities and for localized therapy, for photoacoustic tumor imaging, detection and therapy, and for sonofluorescence tumor imaging, detection and therapy. For example, monooctreotate-bisethylcarboxymethyl indocyanine dye (Cytate 1) was prepd. (yield of 80%) and evaluated in the CA20948 Lewis rat model of pancreatic acinar carcinoma. Using the CCD camera, strong localization of this dye was obsd. in the tumor at 90 min post injection. At 19 h post injection the animal was again imaged and tumor visualization was easily obsd. showing specificity of this agent for somatostatin receptors present in this tumor line.
IT **31362-50-2DP, Bombesin, conjugates** with cyanine dyes
RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**bioconjugates** of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
RN 31362-50-2 HCAPLUS
CN Bombesin (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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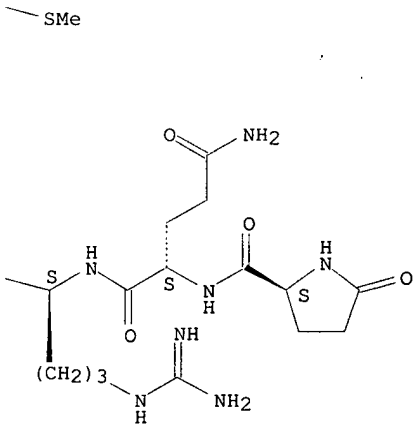

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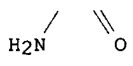

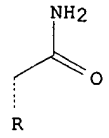

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L39 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2001 ACS
IC ICM A61K041-00
CC 63-8 (Pharmaceuticals)
Section cross-reference(s): 1, 8, 14, 34, 41
ST cyanine dye peptide conjugate imaging diagnosis therapy; tumor imaging therapy cyanine dye peptide conjugate
IT Surgery
(LAGS (laser-assisted guided surgery); bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Pancreas, neoplasm
(acinar cell, adenocarcinoma; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Diagnosis
(agents; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Antiarteriosclerotics
(antiatherosclerotics; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Antitumor agents
Atherosclerosis
Cyanine dyes
Drug delivery systems
Fluorescence
Imaging agents
Light scattering
Optical absorption
Photodynamic therapy
Thrombus
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Somatostatin receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Prostate gland
(carcinoma; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Glycopeptides
RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(conjugates with cyanine dyes; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Peptidomimetics
(conjugates, with cyanine dyes; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Antibodies
Hormones, animal, biological studies
Oligosaccharides, biological studies
Peptides, biological studies
RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(conjugates, with cyanine dyes; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Tomography
(contrast agents; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Imaging agents
(contrast, tomog.; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Pancreas, neoplasm
(duct cell adenocarcinoma; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Imaging
(**fluorescent**, endoscopic; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)
IT Photoacoustic effect
(imaging; bioconjugates of cyanine and indocyanine dyes with peptides

for diagnostic imaging and therapy)

IT Neoplasm
(metastasis, micro-; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

IT Imaging
(photoacustic; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

IT Sonoluminescence
(sonofluorescence; bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

IT 302794-43-0DP, conjugates with cyanine dyes
RL: BPR (Biological process); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

IT 64-17-5, Ethanol, biological studies 67-68-5, Dimethylsulfoxide, biological studies
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

IT 25679-24-7P 83150-76-9P, Octreotide 105466-87-3P 195825-84-4P 302794-43-0P 309916-88-9P 309916-89-0P 309916-90-3P
RL: PNU (Preparation, unclassified); RCT (Reactant); PREP (Preparation)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

IT 590-92-1, 3-Bromopropanoic acid 4224-70-8, 6-Bromohexanoic acid 41532-84-7, 1,1,2-Trimethyl-[1H]-benz[e]indole 309916-91-4 309916-92-5
RL: RCT (Reactant)
(bioconjugates of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

IT 9011-97-6DP, Cholecystokinin, conjugates with cyanine dyes
31362-50-2DP, Bombesin, conjugates with cyanine dyes 37221-79-7DP, Vasoactive intestinal peptide, conjugates with cyanine dyes 39379-15-2DP, Neurotensin, conjugates with cyanine dyes 51110-01-1DP, Somatostatin, conjugates with cyanine dyes 83150-76-9DP, Octreotide, conjugates with cyanine dyes
RL: SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**bioconjugates** of cyanine and indocyanine dyes with peptides for diagnostic imaging and therapy)

=> d bib abs hitstr 3

L39 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:84648 HCAPLUS
DN 132:141941
TI Conjugates and fusion proteins for treating secondary tissue damage and other inflammatory conditions and disorders
IN Mcdonald, John R.; Coggins, Philip J.
PA Osprey Pharmaceuticals Limited, Can.
SO PCT Int. Appl., 204 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000004926	A2	20000203	WO 1999-CA659	19990721
WO 2000004926	A3	20001102		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9948918	A1	20000214	AU 1999-48918	19990721
EP 1098664	A2	20010516	EP 1999-932572	19990721
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI US 1998-120523	A2	19980722		
WO 1999-CA659	W	19990721		

AB Conjugates contg. as a ligand a chemokine receptor-targeting agent, such as chemokines, and a targeted agent, such as a toxin are provided. These conjugates are used to treat inflammatory responses assocd. with activation, proliferation and migration of immune effector cells, including leukocyte cell types, neutrophils, macrophages, and eosinophils. The conjugates provided herein are used to lessen or inhibit these processes to prevent or at least lessen the resulting secondary effects. In particular, the conjugates are used to target toxins to receptors on secondary tissue damage-promoting cells. The ligand moiety can be selected to deliver the cell toxin to such secondary tissue damage-promoting cells as mononuclear phagocytes, leukocytes, natural killer cells, dendritic cells, and T and B lymphocytes, thereby suppressing the proliferation, migration, or physiol. activity of such cells. Among preferred conjugates are fusion proteins having a chemokine, or a biol. active fragment thereof, as the ligand moiety linked to a cell toxin via a peptide linker of from 2 to about 60 amino acid residues.

=> d bib abs hitstr 4

L39 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:427643 HCAPLUS
DN 131:318406
TI A comparative linkage and physical map of bovine chromosome 24 with human chromosome 18
AU Larsen, Niels J.; Hayes, Helen; Bishop, Michael; Davis, Scott K.; Taylor, Jeremy F.; Kirkpatrick, Brian W.
CS Department of Animal Sciences, University of Wisconsin-Madison, Madison, WI, 53706, USA
SO Mamm. Genome (1999), 10(5), 482-487
CODEN: MAMGEC; ISSN: 0938-8990
PB Springer-Verlag New York Inc.
DT Journal
LA English
AB Polymorphic microsatellites have been developed in the vicinity of nine genes on bovine chromosome (BTA) 24, all orthologous to genes on human chromosome (HSA) 18. The microsatellites have been isolated from bacterial and yeast artificial chromosome clones contg. the genes. A linkage map was developed including these polymorphic markers and four anonymous, published microsatellites. Yeast artificial chromosomes contg. six of these genes have also been mapped using **fluorescent** in situ hybridization (FISH), thereby tying the linkage map together with the phys. map of BTA24. Comparing gene location on HSA18 and BTA24 identifies four regions of conserved gene order, each contg. at least two genes. These genes identify six regions of conserved order between human and mouse, two more than in the human-bovine comparison. The breakpoints between regions of conserved order for human-bovine are also breakpoints in the human-mouse comparison. The centromere identifies a fifth conserved region if the BTA24 centromere is orthologous with the HSA18 centromere.
RE.CNT 39
RE
(1) Agaba, M; Mammalian Genome 1997, V8, P530 HCAPLUS
(2) Ballabio, A; Nature Genetics 1993, V3, P277 HCAPLUS
(3) Barendse, W; Mammalian Genome 1997, V8, P21 HCAPLUS
(5) Brown, J; Molecular and Cellular Probes 1995, V9, P53 HCAPLUS
(6) Brownstein, M; BioTechniques 1996, V20, P1004 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 5

L39 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1991:651669 HCAPLUS
DN 115:251669
TI A method for the stepwise, controlled synthesis of chemical species, particularly peptides, on protein substrates, coupled products obtained by the method, and the use of these coupled products, e.g. as vaccines
IN Houen, Gunnar; Holm, Arne
PA Den.
SO PCT Int. Appl., 106 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9108220	A1	19910613	WO 1990-DK311	19901130
	W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, GR, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, RO, SD, SE, SU, US				
	RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG				
	AU 9168929	A1	19910626	AU 1991-68929	19901130
PRAI	DK 1989-6085		19891201		
	WO 1990-DK311		19901130		

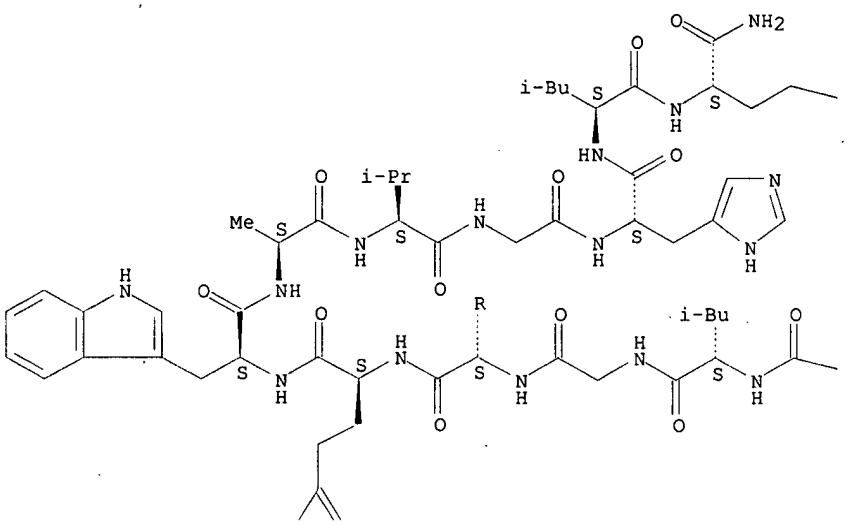
AB Chem. species, esp. peptides, are synthesizied by a stepwise, controlled process using a proteinaceous substances as the synthesis substrate. The coupled products obtained by the process can be used, e.g., as vaccines, matrix materials, or carrier mols. The products, including peptides and peptide derivs., prepd. by the method are also claimed. Bovine serum albumin (BSA) was placed in a silylated reaction vessel and the CO2H groups were diethylamidated before coupling glutamic acid as the Fmoc (9-**fluorenylmethyloxycarbonyl**) and tert-Bu protected Dhbt (3-hydroxy-3,4-dihydrobenzotriazin-4-one ester, blocking remaining amino groups with acetic anhydride, and sequentially coupling Fmoc- and side chain-protected Dhbt esters of lysine, serine, threonine, aspartic acid, methionine, and serine. Piperidine was used to remove the Fmoc protecting group between couplings. Side chain protection groups were removed in CH2Cl2/F3CCO2H (1:1 vol./vol.) at 0.degree.. The product had an av. of 35 synthesized peptide chains per BSA mol. The coupled product was used to raise antibodies to Ser-Met-Asp-Thr-Ser-Lys-Glu in rabbits.

IT **31362-50-2D, Bombesin, conjugates** with protein carrier **102577-19-5D, Neuromedin** B, **conjugates** with protein carrier **102577-22-0D, Neuromedin** C, **conjugates** with protein carrier
RL: RCT (Reactant)
(stepwise synthesis of, for vaccines and other uses)

RN 31362-50-2 HCAPLUS
CN Bombesin (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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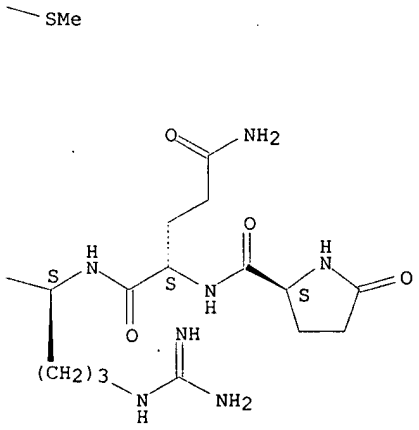

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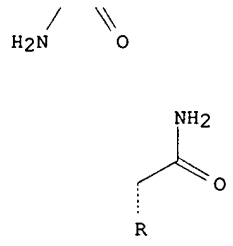


RN 102577-19-5 HCAPLUS
CN Neuromedin B (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RN 102577-22-0 HCAPLUS
CN Neuromedin C (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

=> d bib abs hitstr 6

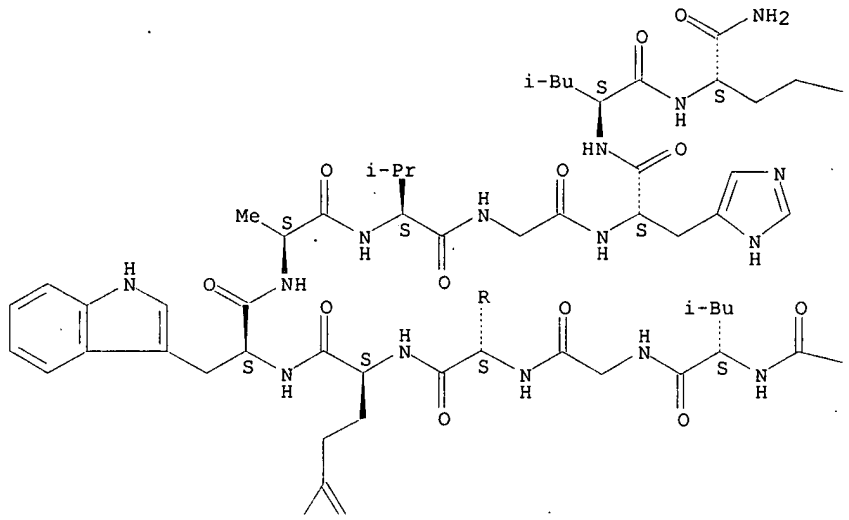
L39 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1991:400778 HCAPLUS
DN 115:778
TI Covalently-linked complexes and methods for enhanced cytotoxicity and imaging
IN Anderson, David C.; Morgan, A. Charles; Abrams, Paul G.; Nichols, Everett J.; Fritzberg, Alan R.
PA NeoRx Corp., USA
SO Eur. Pat. Appl., 23 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 359347	A2	19900321	EP 1989-250014	19890814
	EP 359347	A3	19900418		
	EP 359347	B1	19921223		
	R: AT, BE, CH, DE, ES, FR, GB, GR; IT, LI, LU, NL, SE				
	US 5135736	A	19920804	US 1988-232337	19880815
	US 5169933	A	19921208	US 1989-390241	19890807
	CA 1334513	A1	19950221	CA 1989-608198	19890811
	JP 02124833	A2	19900514	JP 1989-209992	19890814
	AT 83669	E	19930115	AT 1989-250014	19890814
PRAI	US 1988-232337		19880815		
	EP 1989-250014		19890814		

AB Covalently-linked complexes (CLCs) for targeting a defined population of cells comprise a targeting protein (e.g. antibody, hormone, enzyme, etc.), a cytotoxic agent (e.g. radionuclide, toxin, drug, etc.) an enhancing moiety capable of enhancing CLC-target cell interaction (e.g. a translocating/internalizing moiety, an anchoring peptide, membrane-sol. hydrophobic mol., etc.). The CLCs are used to enhance in vivo cytotoxicity and imaging (no data). Translocating peptide, Cys-Gly-Glu-Ala-Ala-Leu-Ala(Glu-Ala-Leu-Ala)4-Glu-Ala-Leu-Glu-Ala-Leu-Ala-Ala-NH2, is conjugated via succinimidyl 4(N-maleimidemethyl)cyclohexane-1-carboxylate (SMCC) to reduced toxin A chain. The conjugate is reacted with iminothiolane to generate further thiol groups which are then bonded to reduced antibody to prep. translocating peptide-ricin A chain-antibody CLC.
IT **31362-50-2D, Bombesin, conjugates** with cytotoxic agent and targeting protein
RL: BIOL (Biological study)
(cell targeting with, for enhanced cytotoxicity and imaging)
RN 31362-50-2 HCAPLUS
CN Bombesin (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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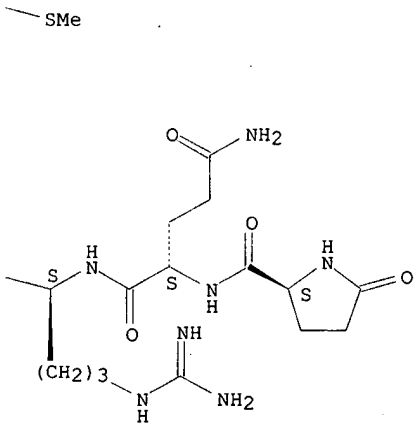

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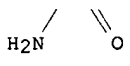

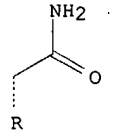

=> d bib abs hitstr 7

L39 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1988:564243 HCAPLUS
DN 109:164243
TI Pathway of phospholipase C activation initiated with platelet-derived growth factor is different from that initiated with vasopressin and bombesin
AU Hasegawa-Sasaki, Hiroko; Lutz, Frieder; Sasaki, Terukatsu
CS Dep. Biochem., Sapporo Med. Coll., Sapporo, 060, Japan
SO J. Biol. Chem. (1988), 263(26), 12970-6
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The effects of GTP, guanosine 5'-O-(2-thiodiphosphate) (GDP.beta.S), and guanosine 5'-O-(3-thiotriphosphate) (GTP.gamma.S) on polyphosphoinositide hydrolysis stimulated by growth factors were studied in rat fibroblast WFB cells made permeable to nucleotides by treatment with either saponin or Pseudomonas aeruginosa cytotoxin. Platelet-derived growth factor (PDGF), vasopressin, and bombesin elicited inositol phosphate prodn. in the permeabilized WFB cells in the absence of added GTP. GDP.beta.S, a competitive inhibitor of GTP-binding proteins (G-proteins), markedly reduced the bombesin- and vasopressin-stimulated prodn. of inositol phosphates. However, the PDGF-stimulated prodn. of inositol phosphates was not affected by the addn. of GDP.beta.S. GTP.gamma.S, an agonist of G-proteins, largely enhanced the vasopressin- and bombesin-stimulated hydrolysis of inositol lipids when added at 10-100 .mu.M. In the presence of GTP.gamma.S, the PDGF-stimulated hydrolysis of inositol lipids was not enhanced, but was reduced: 100 .mu.M GTP.gamma.S reduced the stimulated hydrolysis to about a half of the control level. Only GTP.gamma.S and no other nucleoside triphosphates, had these effects. Activation of G-proteins in WFB cells by **fluoroaluminate** resulted in the inhibition of inositol phosphate prodn. elicited with not only PDGF, but also with vasopressin and bombesin. Thus, a G-protein **couples** vasopressin and **bombesin** receptors to the activation of phospholipase C. Coupling of the PDGF receptor to phospholipase C is not mediated through a G-protein. The results obtained by the use of GTP.gamma.S and NaF plus AlCl3 suggest competition for a phospholipase C between an inhibitory G-protein and a transducer protein constituting the PDGF receptor-linked pathway of phospholipase C activation.

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=> d bib abs hitstr 8
      7 ANSWERS ARE AVAILABLE.  SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE
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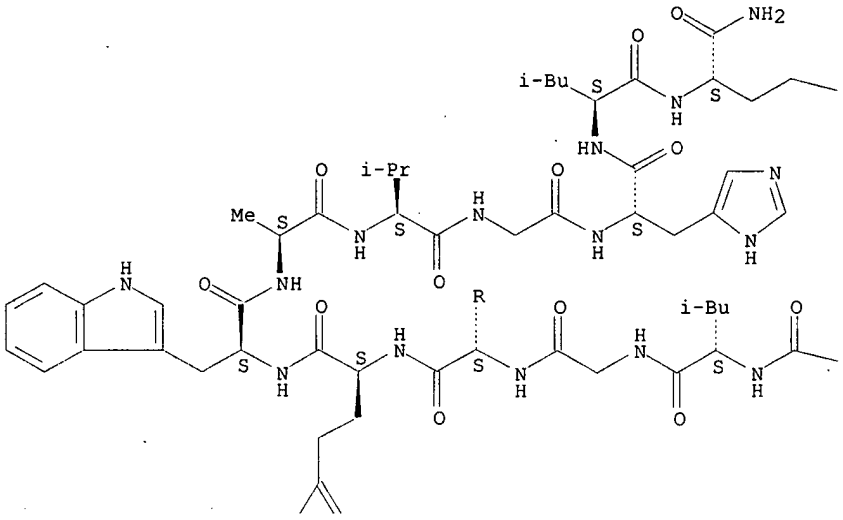
=> d bib abs hitstr

L42 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:181069 HCAPLUS
DN 130:333120
TI Visualization of internalization and recycling of the **gastrin releasing** peptide receptor-green fluorescent protein chimera expressed in epithelial cells
AU Slice, Lee W.; Yee, Hal F., Jr.; Walsh, John H.
CS CURE: VA/UCLA Gastroenteric Biology Center and the Department of Medicine, Division of Digestive Diseases, University of California, Los Angeles, CA, 90095, USA
SO Recept. Channels (1998), 6(3), 201-212, 5 plates
CODEN: RCHAE4; ISSN: 1060-6823
PB Harwood Academic Publishers
DT Journal
LA English
AB **Gastrin releasing** peptide (**GRP**) regulates crit. gastrointestinal functions via the **GRP** receptor (GRPR). GRPR internalization and recycling have been proposed to play an important role in the cellular response to **GRP**. Our aim was to develop a direct method for investigating GRPR trafficking in living cells. A chimeric protein, consisting of GRPR fused to green fluorescent protein (GFP), was expressed in epithelial cells. Ligand and receptor interactions were examd. with radiolabeled agonist and fluorescent imaging. In comparison with epithelial cells expressing wild-type GRPR, the GRPR-GFP expressing cells showed similar ligand binding affinity, **GRP**-stimulated Ca2+ signaling, and **GRP**-initiated internalization. In GRPR-GFP expressing cells treated with fluorescently labeled ligand, receptor and ligand trafficking was directly visualized. Upon ligand binding, the receptor-ligand complex coalesced into vesicles prior to internalization and migration to the perinuclear space. Whereas a portion of the receptors were obsd. to return to the plasma membrane, the ligand remained in the perinuclear space. Hyperosmolar soln. prevented ligand and receptor internalization, and bafilomycin inhibited receptor recycling. We demonstrate that GRPR-GFP is physiol. similar to wild-type GRPR, and permits direct visualization of intracellular trafficking processes in individual living cells with minimal toxicity over several hours.
IT **31362-50-2, Bombesin**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(visualization of internalization and recycling of **gastrin releasing** peptide receptor-green fluorescent protein chimera expressed in epithelial cells)
RN 31362-50-2 HCAPLUS
CN Bombesin (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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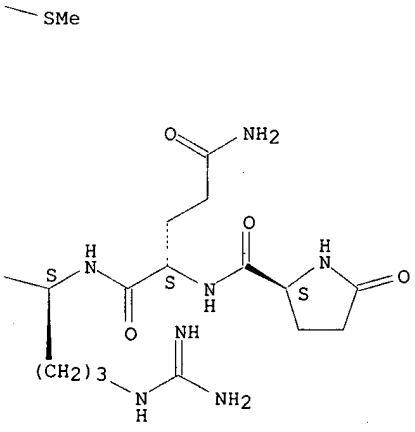

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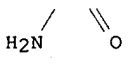

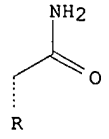

RE.CNT 21
RE
(1) Barak, L; Mol Pharmacol 1997, V51, P177 HCAPLUS
(2) Bowman, E; Proc Natl Acad Sci USA 1988, V85, P7972 HCAPLUS
(3) Doevendans, P; Biochem Biophys Res Commun 1996, V222, P352 HCAPLUS
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(5) Giraud, A; Am J Physiol 1987, V252, PG413 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr l44 1

L44 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:168147 HCAPLUS
DN 130:296981
TI Fluorescent Pseudo-Peptide Linear Vasopressin Antagonists: Design, Synthesis, and Applications
AU Durroux, Thierry; Peter, Marion; Turcatti, Gerardo; Chollet, Andre; Balestre, Marie-Noeelle; Barberis, Claude; Seyer, Rene
CS INSERM U 469 and CNRS UPR 9023, CCIPE, Montpellier, 34094, Fr.
SO J. Med. Chem. (1999), 42(7), 1312-1319
CODEN: JMCMAR; ISSN: 0022-2623
PB American Chemical Society
DT Journal
LA English
AB Fluoresceinyl and rhodamyl groups have been coupled by an **amide** link to side-chain amino groups at positions 1, 6, and 8 of pseudopeptide linear vasopressin antagonists through different positions on the fluorophore, to give tetraethylrhodamyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH2, 4-HOC6H4(CH2)2CO-D-Tyr(Me)-Phe-Gln-Asn-Lys(5-carboxyfluoresceinyl)-Pro-Arg-NH2, 4-HOC6H4(CH2)2CO-D-Tyr(Me)-Phe-Gln-Asn-Lys(5- or 6-carboxytetramethylrhodamyl)-Pro-Arg-NH2, 4-HOC6H4(CH2)2CO-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Lys(5- or 6-carboxyfluoresceinyl)-NH2, 4-HOC6H4(CH2)2CO-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Lys(5-carboxytetramethylrhodamyl)-NH2 (I), and its 6-carboxytetramethylrhodamyl analog. The closer to the C-terminus the fluorophore, the higher the affinities of the fluorescent derivs. for the human vasopressin V1a receptor transfected in CHO cells. Compd. I has a Ki of 70 pM, as detd. by competition expts. with [125I]-4-HOC6H4CH2CO-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH2. It showed a good selectivity for human V1a receptor vs. human oxytocin (Ki = 1.2 nM), human vasopressin V1b (Ki .apprxeq. 27 nM), and human vasopressin V2 (Ki > 5000 nM) receptor subtypes. All fluorescent analogs were antagonists as shown by the inhibition of vasopressin induced inositol phosphate accumulation. These fluorescent ligands are efficient for labeling cells expressing the human V1a receptor subtype, as shown by flow cytofluorometric expts. or fluorescence microscopy. They are also appropriate tools for structural anal. of the vasopressin receptors by fluorescence.
RE.CNT 33
RE
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(2) Carnazzi, E; J Med Chem 1994, V37, P1841 HCAPLUS
(3) Coste, J; Tetrahedron Lett 1990, V31, P205 HCAPLUS
(5) Faure, M; J Histochem Cytochem 1994, V42, P755 HCAPLUS
(6) Frerot, E; Tetrahedron 1991, V47, P259 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr l44 2

L44 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:415460 HCAPLUS
DN 129:172720
TI Ortho-aminobenzoic acid as a fluorescent probe for the interaction between peptides and micelles
AU Turchiello, R. F.; Lamy-Freund, M. T.; Hirata, I. Y.; Juliano, L.; Ito, A. S.
CS Instituto de Fisica, Universidade de Sao Paulo, Sao Paulo, CEP 05315-970, Brazil
SO Biophys. Chem. (1998), 73(3), 217-225
CODEN: BICIAZ; ISSN: 0301-4622
PB Elsevier Science B.V.
DT Journal
LA English
AB The ortho-aminobenzoic acid (o-Abz) has been used as a fluorescent probe in internally quenched fluorescent peptides for continuous protease assays. We investigated the fluorescent properties of the probe in order to verify if it can be used to monitor the interaction of peptides with micelles. Abz-aminoacyl-monomethyl **amide** (Abz-Xaa-NHCH3, where Xaa=Arg, Phe, Leu and Glu) were synthesized. Quantum yield, spectral position, anisotropy and lifetime decay were analyzed in the presence and absence of SDS (SDS) micelles. Significant changes in the fluorescence parameters were obsd. for Abz-Arg-NHCH3 in comparison to Abz-Glu-NHCH3, indicating a strong electrostatic component in the compds. interaction with the neg. charged micelles. The change in fluorescence parameters, obsd. when the probe is bound to hydrophobic amino acids Abz-Phe-NHCH3 and Abz-Leu-NHCH3, is probably due to insertion of those compds. into micelles. Abz-NHCH3 fluorescence is less affected by the presence of micelles, indicating that the occurrence of interaction is dependent on the properties of the aminoacid to which the fluorophore is attached. The quenching data with acrylamide confirmed these results. Titrn. curves allowed the estn. of assocn. consts. between Abz compds. and SDS, according to a single partition model. Although the results cannot be strictly applied to the titrn. with charged compds., it was verified that the assocn. const. for the isolated Abz-NHCH3 is significantly lower than those for Abz-Phe-NHCH3 and Abz-Leu-NHCH3. It is concluded that the Abz group is a sensitive and convenient fluorescent probe to monitor peptide binding to amphiphilic aggregates. That conclusion is supported by measurements with the peptide Abz-Leu-Arg-Phe-NH2.

=> d bib abs hitstr 1

L48 ANSWER 1 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:719189 HCAPLUS
DN 133:344747
TI A combination assay for simultaneous assessment of multiple signaling pathways
AU Goetz, A. S.; Liacos, J.; Yingling, J.; Ignar, D. M.
CS Department of Receptor Biochemistry, Glaxo Wellcome Research and Development, Research Triangle Park, NC, 27709, USA
SO J. Pharmacol. Toxicol. Methods (**1999**), 42(4), 225-235
CODEN: JPTMEZ; ISSN: 1056-8719
PB Elsevier Science Inc.
DT Journal
LA English
AB The authors have developed an assay in which modulation of two or more signaling pathways can be assessed concurrently by combining reporter gene systems with fluorescent probe technol. The validation of this method was achieved by indirect anal. of adenylyl cyclase activation with the use of a cAMP response element (CRE)-luciferase reporter system in combination with the measurement of calcium mobilization by Calcium Green-1 AM fluorescence on a fluorescent imaging plate reader. To demonstrate the utility of the method in studying the pharmacol. of receptors that **couple** to more than one G protein, Chinese hamster ovary (CHO) cells, which stably expressed both the CRE-luciferase reporter gene and the human pituitary adenylyl cyclase-activating peptide (PACAP) receptor, were treated with PACAP 1-27 and 1-38. Calcium mobilization and the induction of adenylyl cyclase activity in response to each concn. of peptide were assessed in individuals wells. This assay may also be used to screen for ligands of two or more unrelated receptors simultaneously without compromising the assessment of either signaling pathway. To illustrate this point, Rat-1 fibroblasts, which expressed human .alpha.1A receptors, were cocultured with CRE-luciferase CHO cells, which expressed human GLP-1 receptors. Calcium mobilization elicited by phenylephrine agonism of the .alpha.1A receptor was assessed in the same assay as GLP-1-induced activation of adenylyl cyclase. The pEC50 for each agonist was similar to that obsd. when the cell lines were not cocultured. The no. of different receptors that can be screened per well is limited only by the ability to distinguish different reporter gene signals and fluorescent indicators.
RE.CNT 23
RE
(1) Basille, M; J Neurochem 1995, V65, P1318 HCAPLUS
(2) Bevan, N; Neuroreport 1998, V9, P2703 HCAPLUS
(4) Cheng, Y; Biochem Pharmacol 1973, V22, P3099 HCAPLUS
(5) Ciccarelli, E; Eur J Pharmcol 1995, V288, P259 HCAPLUS
(6) Delporte, C; Mol Cell Endocrinol 1995, V107, P71 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 2

L48 ANSWER 2 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:658520 HCAPLUS
DN 133:251273
TI Metal-lipid molecules
IN Hainfeld, James F.; Furuya, Frederic R.; Powell, Richard D.; Joshi, Vishwas N.; Gutierrez, Edmund
PA Nanoprobes, Inc., USA
SO U.S., 20 pp., Cont.-in-part of U.S. 5,728,590.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6121425	A	20000919	US 1998-39601	19980316
	US 5521289	A	19960528	US 1994-282929	19940729 <--
	US 5728590	A	19980317	US 1996-652007	19960523 <--
PRAI	US 1994-282929	A2	19940729		
	US 1996-652007	A2	19960523		

AB Disclosed are novel metal-lipid mols. for **conjugating** antibody (or protein or nucleic acid) for diagnostic and therapeutic uses. The metal-lipid mols. comprise a cluster or colloid of atoms of Au, Ag, Pt, Pd, or combinations thereof, an org. group **covalently attached** to the metal atoms, and a lipid moiety. In a preferred embodiment, the metal cluster has about 50-70 gold atoms and a diam. of about 1.4 nm and the lipid moiety is dipalmitoyl phosphatidyl ethanolamine.

RE.CNT 19
RE
(1) Anon; GB 1214552 1970 HCAPLUS
(6) Brust, M; J Chem Soc, Chem Comm 1994, P801 HCAPLUS
(7) Danielmeyer; US 4124524 1978 HCAPLUS
(14) Klainer; US 5116759 1992 HCAPLUS
(15) Leuvering; US 4313734 1982 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 3

L48 ANSWER 3 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:365078 HCAPLUS
DN 131:211255
TI Application of donor-donor energy migration (DDEM) for examining protein structure and function
AU Bergstroem, Fredrik; Haggloef, Peter; Karolin, Jan; Ny, Tor; Johansson, Lennart B.-A.
CS Dep. Physical Chemistry, Umea Univ., Umea, Swed.
SO Proc. SPIE-Int. Soc. Opt. Eng. (**1999**), 3602(Advances in Fluorescence Sensing Technology IV), 250-255
CODEN: PSISDG; ISSN: 0277-786X
PB SPIE-The International Society for Optical Engineering
DT Journal
LA English
AB Donor-Donor Energy Migration (DDEM) and fluorescence anisotropy expts. can be utilized as a versatile tool for examg. protein structure and function. For this, pairs of identical fluorescent probes (D) are **attached** to unique residues created by means of site specific mutagenesis. Present work illustrates the applicability of the method on the latent form of Plasminogen Activator Inhibitor-1 (PAI-1). Different DD-pairs of mutated PAI-1 were prepd. and studied, namely; V106C-H185C, H185C-M266C and M266C-V106C. The Cys residues were labeled with a sulfhydryl specific deriv. of BODIPY [N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-yl)methyl iodo-acetamide]. To det. the rate of DDEM within such a pair, intramol. order and dynamics must be considered. For anal. of data, addnl. information was obtained from expts. with the corresponding D-labeled single Cys mutants, i.e., V106C, H185C and M266C. The stability of values detd. was tested by generating and re-analyzing synthetic data. The intramol. distances obtained agree, reasonably well, with those detd. from the x-ray structure of latent PAI-1.
RE.CNT 11
RE
(1) Aleshkov, S; J Biol Chem 1996, V271, P21231 HCAPLUS
(4) Forster, T; Ann Phys 1948, V2, P55 HCAPLUS
(5) Johansson, L; J Chem Soc Faraday Trans 1996, V92, P1563 HCAPLUS
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 4

L48 ANSWER 4 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:56154 HCAPLUS
DN 130:264230
TI Live confocal analysis with fluorescently labeled proteins
AU Francis-Lang, Helen; Minden, Jonathan; Sullivan, William; Oegema, Karen
CS Department of Biology, University of California, Santa Cruz, CA, USA
SO Methods Mol. Biol. (Totowa, N. J.) (**1999**), 122(Confocal Microscopy Methods and Protocols), 223-239
CODEN: MMBIED; ISSN: 1064-3745
PB Humana Press Inc.
DT Journal
LA English
AB A protocol for directly **attaching** fluorescent probes to antibodies for live anal. and the application of this procedure more generally to purified and bacterially overexpressed proteins was described. General principles of microinjecting fluorescently labeled proteins, using Drosophila embryos as a specific example was presented as well. This technique was successfully applied in Drosophila and other model organisms.
RE.CNT 24
RE
(2) Debec, A; J Cell Biol 1996, V134, P103 HCAPLUS
(3) Fogarty, P; Development 1994, V120, P2131 HCAPLUS
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(7) Hird, S; J Cell Sci 1996, V109, P525 HCAPLUS
(8) Hyman, A; Methods Enzymol 1991, V196, P478 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 5

L48 ANSWER 5 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:793060 HCAPLUS
DN 130:57170
TI Liposomal **conjugated** peptide nucleic acids having enhanced cellular uptake
IN Nielsen, Peter E.; Knudsen, Helle
PA Isis Pharmaceuticals, Inc., USA
SO PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	WO 9853801	A1	19981203	WO 1998-US10804	19980528	<--
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	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
	AU 9876021	A1	19981230	AU 1998-76021	19980528	<--
	EP 1003480	A1	20000531	EP 1998-923819	19980528	
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
	JP 2001501975	T2	20010213	JP 1999-500871	19980528	
PRAI	US 1997-864765	A	19970528			
	WO 1998-US10804	W	19980528			

OS MARPAT 130:57170
AB Peptide nucleic acids **conjugated** to lipophilic groups and incorporated into liposomes exhibit enhanced cellular uptake and distribution. Cellular uptake and distribution of peptide nucleic acids also increases with the introduction of an amino acid side chain into the backbone of peptide nucleic acids. Methods of modulating cellular uptake and methods for treating animals are provided. The peptide nucleic acids of the invention comprise naturally-occurring nucleobases and non-naturally-occurring nucleobases **attached** to a polyamide backbone.
RE.CNT 3
RE
(1) Anon; Pierce ImmunoTechnology Catalog and Handbook 1992, PE65
(2) Lansdorp; WO 9714026 A2 1997 HCAPLUS
(3) Nielsen; US 5539082 A 1996 HCAPLUS

=> d bib abs hitstr 6

L48 ANSWER 6 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:725811 HCAPLUS
DN 130:1900
TI Fluorescent speckle microscopy, a method to visualize the dynamics of protein assemblies in living cells
AU Waterman-Storer, Clare M.; Desai, Arshad; Bulinski, J. Chloe; Salmon, E. D.
CS Department of Biology, University of North Carolina, Chapel Hill, NC, 27599-3280, USA
SO Curr. Biol. (**1998**), 8(22), 1227-1230
CODEN: CUBLE2; ISSN: 0960-9822
PB Current Biology Publications
DT Journal
LA English
AB Fluorescence microscopic visualization of fluorophore-**conjugated** proteins that have been microinjected or expressed in living cells and have incorporated into cellular structures has yielded much information about protein localization and dynamics. This approach has, however, been limited by high background fluorescence and the difficulty of detecting movement of fluorescent structures because of uniform labeling. These problems have been partially alleviated by the use of more cumbersome methods such as three-dimensional confocal microscopy, laser photobleaching and photoactivation of fluorescence. We report here a method called fluorescent speckle microscopy (FSM) that uses a very low concn. of fluorescent subunits, conventional wide-field fluorescence light microscopy and digital imaging with a low-noise, cooled charged **coupled** device (CCD) camera. A unique feature of this method is that it reveals the assembly dynamics, movement and turnover of protein assemblies throughout the image field of view at diffraction-limited resoln. We found that FSM also significantly reduces out-of-focus fluorescence and greatly improves visibility of fluorescently labeled structures and their dynamics in thick regions of living cells. Our initial applications include the measurement of microtubule movements in mitotic spindles and actin retrograde flow in migrating cells.
RE.CNT 15
RE
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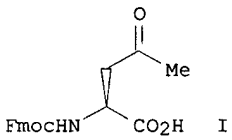
(3) Desai, A; J Cell Biol 1998, V141, P703 HCAPLUS
(4) Hyman, A; Meth Enzymol 1991, V196, P478 HCAPLUS
(5) Parsons, S; Cell Motil Cytoskeleton 1997, V36, P1 HCAPLUS
(7) Sase, I; Biophys J 1995, V69, P323 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 7

L48 ANSWER 7 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:625026 HCAPLUS
DN 129:302877
TI Direct incorporation of unprotected ketone groups into peptides during solid-phase synthesis: application to the one-step **modification** of peptides with two different biophysical probes for FRET
AU Marcaurelle, Lisa A.; Bertozzi, Carolyn R.
CS Dep. Chem., Univ. California, Berkeley, CA, 94720, USA
SO Tetrahedron Lett. (**1998**), 39(40), 7279-7282
CODEN: TELEAY; ISSN: 0040-4039
PB Elsevier Science Ltd.
DT Journal
LA English
GI



I

AB Protected (2S)-aminolevulinic acid I (Fmoc = 9-fluorenylmethoxycarbonyl), bearing an unprotected ketone group, was incorporated into a synthetic peptide using std. Fmoc-based solid-phase methods. The ketone group remained unharmed during the synthesis and provided a uniquely reactive functional group for **covalent modification** of the peptide. The ketone and a cysteine sulfhydryl group elsewhere in the peptide were reacted simultaneously with two different biophys. probes, enabling the site-specific installation of a donor and acceptor pair for fluorescence resonance energy transfer (FRET) in one step without the need for differential side chain protection.

=> d bib abs hitstr 8

L48 ANSWER 8 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:572338 HCAPLUS
DN 129:186415
TI Detection of targets with green fluorescent protein and fluorescent variants thereof
IN Plaia, Todd W.
PA Oncor, Inc., USA
SO PCT Int. Appl., 32 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	WO 9836099	A1	19980820	WO 1998-US3147	19980218	<--
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	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
	AU 9866588	A1	19980908	AU 1998-66588	19980218	<--
	EP 1000172	A1	20000517	EP 1998-908588	19980218	
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
PRAI	US 1997-38623	P	19970218			
	WO 1998-US3147	W	19980218			

AB A labeled marker for detection of a target is described which includes a label selected from the group consisting of green fluorescent protein and a fluorescent variant thereof, and a ligand configured to bind to the target. The ligand includes any mol. or combination of mols. which have an affinity for another substance. For example, the ligand can be selected from the group consisting of nucleic acid probe, antibody, hapten **conjugate**, biotin, avidin and streptavidin. A method for detecting a target is also described which includes providing a primary ligand configured to bind to the target and providing a secondary ligand configured to bind to the primary ligand. Techniques such as fluorescent microscopy are used to visualize the labeled marker.

=> d bib abs hitstr 9

L48 ANSWER 9 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:496687 HCAPLUS
DN 129:227435
TI TNP-ATP and TNP-ADP as probes of the nucleotide binding site of CheA, the histidine protein kinase in the chemotaxis signal transduction pathway of Escherichia coli
AU Stewart, Richard C.; VanBruggen, Ricaele; Ellefson, Dolph D.; Wolfe, Alan J.
CS Department of Cell Biology Molecular Genetics, University of Maryland, College Park, MD, 20742, USA
SO Biochemistry (**1998**), 37(35), 12269-12279
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
AB The interaction of CheA with ATP has important consequences in the chemotaxis signal transduction pathway of Escherichia coli. This interaction results in autophosphorylation of CheA, a histidine protein kinase. Autophosphorylation of CheA sets in motion a chain of biochem. events that enables the chemotaxis receptor proteins to communicate with the flagellar motors. As a result of this communication, CheA allows the receptors to control the cell swimming pattern in response to gradients of attractant and repellent chems. To probe CheA interactions with ATP, we investigated the interaction of CheA with the fluorescent nucleotide analogs TNP-ATP [2'(3')-O-(2,4,6-trinitrophenyl)ATP] and TNP-ADP. Spectroscopic studies indicated that CheA bound TNP-ATP and TNP-ADP with high affinity (micromolar Kd values) and caused a marked enhancement of the fluorescence of the TNP moiety of these **modified** nucleotides. Anal. of titrn. expts. indicated a binding stoichiometry of two mols. of TNP-ATP (TNP-ADP) per CheA dimer and suggested that the two binding sites on the CheA dimer operate independently. Binding of TNP-ATP to CheA was inhibited by ATP, and anal. of this inhibition indicated that the CheA dimer binds 2 mols. of ATP. Competition expts. also indicated that CheA binds TNP-ATP considerably more tightly than it binds unmodified ATP. Binding of TNP-ADP to CheA was inhibited by ADP in a similar manner. TNP-ATP was not a substrate for CheA and served as a potent inhibitor of CheA autophosphorylation (Ki < 1 .mu.M). The glycine-rich regions (G1 and G2) of CheA and other histidine protein kinases have been presumed to play important roles in ATP binding and/or catalysis of CheA autophosphorylation, although few exptl. tests of these functional assignments have been made. Here, we demonstrate that a CheA mutant protein with Gly.fwdarw.Ala substitutions in G1 and G2 has a markedly reduced affinity for ATP and ADP, as measured by Hummel-Dreyer chromatog. This mutant protein also bound TNP-ATP and TNP-ADP very poorly and had no detectable autokinase activity. Surprisingly, a distinct single-site substitution in G2 (Gly470.fwdarw.Lys) had no observable effect on the affinity of CheA for ATP and ADP, despite the fact that it rendered CheA completely inactive as an autokinase. This mutant protein also bound TNP-ATP and TNP-ADP with affinities and stoichiometries that were indistinguishable from those obsd. with wild-type CheA. These results provide some preliminary insight into the possible functional roles of G1 and G2, and they suggest that TNP-nucleotides are useful tools for exploring the effects of addnl. mutations on the active site of CheA.

=> d bib abs hitstr 10

L48 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:425671 HCAPLUS
DN 129:158836
TI Strategies for positioning fluorescent probes and crosslinkers on formyl peptide ligands
AU Vilven, Janeen C.; Domalewski, Mark; Prossnitz, Eric R.; Ye, Richard D.; Muthukumaraswamy, Natesa; Harris, Robert B.; Freer, Richard J.; Sklar, Larry A.
CS Cancer Center and Pathology, Univ. New Mexico Health Sciences Cent., Albuquerque, NM, USA
SO J. Recept. Signal Transduction Res. (**1998**), 18(2 & 3), 187-221
CODEN: JRETET; ISSN: 1079-9893
PB Marcel Dekker, Inc.
DT Journal
LA English
AB Chemoattractant receptors represent a major subset of the G-protein **coupled** receptor (GPCR) family. One of the best characterized, the N-formyl peptide receptor (FPR), participates in host defense responses of neutrophils. The features of the ligand which regulate its interaction with the FPR are well-known. By manipulating these features we have developed new ligands to probe structural and mechanistic aspects of the peptide-receptor interaction. Three ligand groups have bee developed: (1) ligands contg. a Lys residue located in positions 2 through 7 that can be **conjugated** to FITC (N-formyl-Met1-Lys2-Phe3-Phe4, N-formyl-Met1-Leu2-Lys3-Phe4, N-formyl-Met1-Leu2-Phe3-Lys4, N-formyl-Met1-Leu2-Phe3-Phe4-Lys5, N-formyl-nLeu1-Leu2-Phe3-nLeu4-Tyr5-Lys6 and N-formyl-Met1-Leu2-Phe3-Phe4-Gly5-Gly6-Lys7); (2) fluorescent pentapeptide ligands (N-formyl-Met-X-Phe-Phe-Lys(FITC) where X = Leu, Ala, Val or Gly); and (3) small crosslinking ligands where the photoaffinity crosslinker 4-azidosalicylic acid (ASA) was **conjugated** to Lys is positions 3 and 4 and p-benzoyl-phenylalanine (Bpa) was located in position 2 in N-formyl-Met1-Bpa2-Phe3-Tyr4. The peptides were characterized according to activity and affinity in human neutrophils and cell lines transfected with FPR. All of the peptides were agonists, with parallel affinity and activity. In the fast group, the peptide activity decreases as Lys is placed closer to the N-formyl group and the activity is improved by 1-3 orders of magnitude by **conjugation** with FITC. In the second group, the dissocn. rate of the peptide from the receptor increases as position 2 is replaced by aliph. amino acids with smaller alkyl groups. In the third group, crosslinking ligands remain biol. active, display nM affinity and **covalently** label the FPR.

=> d bib abs hitstr 11

L48 ANSWER 11 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:296507 HCAPLUS
DN 129:52466
TI Visualization of dynamic trafficking of a protein kinase C .beta.II/green fluorescent protein **conjugate** reveals differences in G protein-**coupled** receptor activation and desensitization
AU Feng, Xiao; Zhang, Jie; Barak, Larry S.; Meyer, Tobias; Caron, Marc G.; Hannun, Yusuf A.
CS Department of Cell Biology, Duke University Medical Center, Durham, NC, 27710, USA
SO J. Biol. Chem. (**1998**), 273(17), 10755-10762
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB Protein kinase C (PKC) **links** various extracellular signals to intracellular responses and is activated by diverse intracellular factors including diacylglycerol, Ca2+, and arachidonic acid. In this study, using a fully functional green fluorescent protein-**conjugated** PKC.beta.II (GFP-PKC.beta.II), we demonstrate a novel approach to study the dynamic redistribution of PKC in live cells in response to G protein-**coupled** receptor activation. Agonist-induced PKC translocation was rapid, transient, and selectively mediated by the activation of Gq.alpha.- but not Gs.alpha.- or Gi.alpha.-**coupled** receptors. Interestingly, although the stimuli were continuously present, only one brief peak of PKC membrane translocation was obsd., consistent with rapid desensitization of the signaling pathway. Moreover, when GFP-PKC.beta.II was used to examine cross-talk between two Gq.alpha.-**coupled** receptors, angiotensin II type 1A receptor (AT1AR) and endothelin A receptor (ETAR), activation of ETARs resulted in a subsequent loss of AT1AR responsiveness, whereas stimulation of AT1ARs did not cause desensitization of the ETAR signaling. The development of GFP-PKC.beta.II has allowed not only the real time visualization of the dynamic PKC trafficking in live cells in response to physiol. stimuli but has also provided a direct and sensitive means in the assessment of activation and desensitization of receptors implicated in the phospholipase C signaling pathway.

=> d bib abs hitstr 12

L48 ANSWER 12 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:159538 HCAPLUS
DN 128:280469
TI Detection and quantitation of heterotrimeric G proteins by fluorescence resonance energy transfer
AU Remmers, Ann E.
CS Department of Pharmacology, The University of Michigan, Ann Arbor, MI, 48109-0632, USA
SO Anal. Biochem. (**1998**), 257(1), 89-94
CODEN: ANBCA2; ISSN: 0003-2697
PB Academic Press
DT Journal
LA English
AB N-Methyl-3'-O-anthranoyl (mant) guanine nucleotide analogs are useful environmentally sensitive fluorescent probes for detection of heterotrimeric guanine nucleotide binding proteins. The mant deriv. of GTP.gamma.S, mGTP.gamma.S, is synthesized and purified by **modification** of a method initially described by T. Hiratsuka (1983). The binding affinity of mGTP.gamma.S for G proteins Gi and Go is comparable to that of GTP.gamma.S. The rate of binding is detd. by the dissocn. rate of the endogenously bound GDP. The large fluorescence increase obsd. upon mGTP.gamma.S binding to G protein is due, in part, to resonance energy transfer from tryptophans in the G protein to the mant guanine nucleotide. The magnitude of the fluorescence increase is dependent upon the concn. of G protein. Therefore, mGTP.gamma.S binding can be used to quantitate and locate G proteins during the protein purifn. process. This method is rapid compared to the [35S]GTP.gamma.S binding assay in that (i) the bound ligand does not need to be sepd. from the free ligand thus avoiding vacuum filtration and (ii) the time required to measure fluorescence in each sample is less than that required for scintillation counting. In addn., the use of radioactivity can be avoided. Thus, the mGTP.gamma.S binding assay for the detection of Gi and Go represents a rapid, reliable alternative to assays based on radiolabeled GTP.gamma.S binding or ADP-ribosylation with pertussis toxin.

=> d bib abs hitstr 13

L48 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:772086 HCAPLUS
DN 128:112486
TI The phytofluors: a new class of fluorescent protein probes
AU Murphy, John T.; Lagarias, J. Clark
CS Section of Molecular and Cellular Biology, University of California, Davis, CA, 9561 6, USA
SO Curr. Biol. (1997), 7(11), 870-876
CODEN: CUBLE2; ISSN: 0960-9822
PB Current Biology Ltd.
DT Journal
LA English
AB Biol. compatible fluorescent protein probes, particularly the self-assembling green fluorescent protein (GFP) from the jellyfish Aequorea victoria, have revolutionized research in cell, mol. and developmental biol. because they allow visualization of biochem. events in living cells. Addnl. fluorescent proteins that could be reconstituted in vivo while extending the useful wavelength range towards the orange and red regions of the light spectrum would increase the range of applications currently available with fluorescent protein probes. Intensely orange fluorescent adducts, which we designate phytofluors, are spontaneously formed upon incubation of recombinant plant phytochrome apoproteins with phycoerythrobilin, the linear tetrapyrrole precursor of the phycoerythrin chromophore. Phytofluors have large molar absorption coeffs., fluorescence quantum yields greater than 0.7, excellent photostability, stability over a wide range of pH, and can be reconstituted in living plant cells. The phytofluors constitute a new class of fluorophore that can potentially be produced upon bilin uptake by any living cell expressing an apophytochrome cDNA. Mutagenesis of the phytochrome apoprotein and/or alteration of the linear tetrapyrrole precursor by chem. synthesis are expected to afford new phytofluors with fluorescence excitation and emission spectra spanning the visible to near-IR light spectrum.

=> d bib abs hitstr 14

L48 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:332770 HCAPLUS
DN 127:62698
TI Detection in living cells of Ca2+-dependent changes in the fluorescence emission of an indicator composed of two green fluorescent protein variants **linked** by a calmodulin-binding sequence. A new class of fluorescent indicators
AU Romoser, Valerie A.; Hinkle, Patricia M.; Persechini, Anthony
CS Department Pharmacology Physiology, University Rochester Medical Center, Rochester, NY, 14642, USA
SO J. Biol. Chem. (**1997**), 272(20), 13270-13274
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB We have designed a novel fluorescent indicator composed of two green fluorescent protein variants joined by the calmodulin-binding domain from smooth muscle myosin light chain kinase. When (Ca2+)4-calmodulin is bound to the indicator (Kd = 0.4 nM), fluorescence resonance energy transfer between the two fluorophores is attenuated; the ratio of the fluorescence intensity measured at 505 nm to the intensity measured at 440 nm decreases 6-fold. Images of microinjected living cells demonstrate that emission ratios can be used to monitor spatio-temporal changes in the fluorescence of the indicator. Changes in indicator fluorescence in these cells are **coupled** with no discernible lag (<1 s) to changes in the cytosolic free Ca2+ ion concn., ranging from below 50 nM to .apprx.1 .mu.M. This observation suggests that the activity of a calmodulin target with a typical 1 nM affinity for (Ca2+)4-calmodulin is responsive to changes in the intracellular Ca2+ concn. over the physiol. range. It is likely that the indicator we describe can be **modified** to detect the levels of ligands and proteins in the cell other than calmodulin.

=> d bib abs hitstr 15

L48 ANSWER 15 OF 15 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:114489 HCAPLUS
DN 126:222460
TI Fluorescence spectroscopy as a tool to investigate protein interactions
AU Brown, Martha P.; Royer, Catherine
CS School of Pharmacy, University of Wisconsin-Madison, Madison, WI, 53706, USA
SO Curr. Opin. Biotechnol. (**1997**), 8(1), 45-49
CODEN: CUOBE3; ISSN: 0958-1669
PB Current Biology
DT Journal
LA English
AB Recent advances in the use of fluorescence spectroscopy to study protein interactions have primarily involved combinations of classic fluorescence techniques, novel probe and **coupling** chemistries, and advances in laser excitation and detection capabilities. For example, new **coupling** strategies for fluorescent probes have allowed the first detn. of the .DELTA.G* describing the insertion of a protein into a membrane. Fluorescently labeled oligonucleotides with specific protein-binding sequences have been used to study both protein-DNA assocns. and oligonucleotide hybridization using anisotropy changes. The first kinetic data describing a DNA-protein binding event was collected with stopped-flow fluorescence instrumentation. Combining scanning fluctuation correlation spectroscopy with a two-photon excitation source improved this technique so that it may now be used to study protein self-assocns.

=> d bib abs 1

L51 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:78252 HCAPLUS
DN 126:180823
TI Design, synthesis, and in vitro evaluation of cytotoxic analogs of bombesin-like peptides containing doxorubicin or its intensely potent derivative, 2-pyrrolinodoxorubicin
AU Nagy, Attila; Armatis, Patricia; Cai, Ren-Zhi; Szepeshazi, Karoly; Halmos, Gabor; Schally, Andrew W.
CS Endocrine, Polypeptide Cancer Institute, Tulane University School Medicine, New Orleans, LA, 70146, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(2), 652-656
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB Five peptide fragments, based on the C-terminal sequence of **bombesin** (BN)-(6-14) or BN-(7-14), were selected as carriers for radicals doxorubicin (DOX) and 2-pyrrolino-DOX to create hybrid cytotoxic analogs. All these compds. had a reduced **peptide bond** (CH2-NH or CH2-N) between positions 13 (Phe or Leu) and 14 (Phe, Leu, or Tac) (Tac = thiazolidine-4-carboxylic acid). Three pseudononapeptide carriers contained N-terminal D-Phe or D-Tpi at position 6 (Tpi = 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid). Two pseudooctapeptides had Gln7 at the N terminus. The conjugation of N-(9-**fluorenylmethoxycarbonyl**)doxorubicin (N-Fmoc-DOX)-14-O-hemiglutarate to the peptide carriers at the N terminus resulted in cytotoxic hybrids of BN-like peptides contg. DOX. These hybrids could then be converted to analogs with 2-pyrrolino-DOX by a reaction with 4-iodobutyraldehyde. The ability of the carriers and the conjugates to inhibit the binding of 125I-labeled [Tyr4]BN to receptors for BN/**gastrin releasing** peptide (**GRP**) on Swiss 3T3 cells was detd. Cytotoxic conjugates of pseudooctapeptide carrier analogs displayed the highest binding affinity (KD .apprxeq.1 nM). The cytotoxic BN analogs and their corresponding cytotoxic radicals exerted similar inhibitory effects on the in vitro growth of CFPAC-1 human pancreatic cancer, DMS-53 human lung cancer, PC-3 human prostate cancer, and MKN-45 human gastric cancer cell lines that have receptors for BN/**GRP**. In DMS-53 cells, the activity of 2-pyrrolino-DOX and its conjugates was .apprxeq.2500 times higher than that of DOX and its hybrids. These highly potent cytotoxic analogs of BN have been designed as targeted anti-tumor agents for the treatment of various cancers that possess receptors for BN/**GRP**.

=> d bib abs 2

L51 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1995:922486 HCAPLUS
DN 124:117903
TI Novel Carboxylic Acid and Carboxamide Protective Groups Based on the Exceptional Stabilization of the Cyclopropylmethyl Cation
AU Carpino, Louis A.; Chao, Hann Guang; Ghassemi, Shahnaz; Mansour, E. M. E.; Riemer, Christoph; Warrass, Ralf; Sadat-Aalaee, Dean; Truran, George A.; Imazumi, Hideko; et al.
CS Wenschuh, Holger, Department of Chemistry, University of Massachusetts, Amherst, MA, 01003-4510, USA; Beyermann, Michael; Bienert, Michael; Shroff, Hitesh; Albericio, Fernando; Triolo, Salvatore A.; Sole, Nuria A.; Kates, Steven A.
SO J. Org. Chem. (1995), 60(24), 7718-19
CODEN: JOCEAH; ISSN: 0022-3263
DT Journal
LA English
OS CASREACT 124:117903
AB The secondary dicyclopropylmethyl (Dcpm) group has been shown to be useful for protection of the carboxylic acid function of peptides in cases where selective deblocking in the presence of tert-Bu based or trityl side chain protection is desired. Examples involved synthesis of protected tripeptide Fmoc-Gly-Asp(OCMe3)-Ala-OH (Fmoc = 9-**fluorenylmethoxycarbonyl**) and hexapeptide Fmoc-Ile-Thr(CMe3)-Arg(Pmc)-Gln(CPh3)-Arg(Pmc)-Tyr(CMe3)-OH (Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl), both of which were assembled by soln. methods. Comparable **amide** protection was possible via the dimethylcyclopropylmethyl (Dmcp) residue which could be deblocked along with tert-Bu-based side chain protecting groups by CF3CO2H. The new Dmcp group was used for protection of Asn and Gln during the solid phase syntheses of a no. of peptides contg. these two amino acids [acyl carrier protein(65-74), **bombesin**, etc.]. Dmcp protection of the C-terminal **amide** function allowed development of a novel direct synthesis of peptide **amides** by the rapid soln. approach. No evidence was obtained for retention of isomeric Dcpm or Dmcp residues during the final deblocking step thus showing that no unproductive ring expansion rearrangement occurred in the presence of acid.

=> d bib abs 3

L51 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1994:474075 HCAPLUS
DN 121:74075
TI Potent pseudopeptide bombesin-like agonists and antagonists: correlation of ordered conformation of bombesin analogs to receptor activity
AU Edwards, J. Vincent; McLean, Larry R.; Wade, Arlene C.; Eaton, Scott R.; Cashman, Elizabeth A.; Hagaman, Karen A.; Fanger, Bradford O.
CS Marion Merrell Dow Res. Inst., Cincinnati, OH, USA
SO Int. J. Pept. Protein Res. (1994), 43(4), 374-83
CODEN: IJPPC3; ISSN: 0367-8377
DT Journal
LA English
AB **Bombesin**-like pseudopeptides have been synthesized, and certain physicochem. properties and biol. activities have been examd. **Bombesin** and the related peptide litorin were modified at positions 13-14 and 8-9, resp., with .psi.[CH2S] and .psi.[CH2N(CH3)]. [Phe13.psi.[CH2S]Leu14]**bombesin** and [Phe8.psi.[CH2S]-Leu9]litorin bound to the murine pancreatic **bombesin/gastrin-releasing** peptide receptor with similar dissocn. consts. (Kd = 3.9 and 3.4 nM, resp.). Increased potency was achieved by oxidn. of the thiomethylene ether to two diastereomeric sulfoxides (isomer I, Kd = 1.6 nM and isomer II, Kd = 0.89 nM). Further oxidn. to the sulfone decreased potency ([Phe8.psi.[CH2SO2]Leu9]litorin, Kd = 9.9 nM). All five analogs were receptor antagonists as detd. by phosphatidylinositol turnover in murine pancreas. In contrast to these peptide backbone substitutions, a .psi.[CH2N(CH3)] at the 8-9 **amide** bond position resulted in an agonist. The analogs were compared with those of litorin (Kd = 0.1 nM) and [Leu9]litorin (Kd = 0.17 nM) by CD and **fluorescence** spectroscopy. The CD spectra demonstrated ordered conformation for all the peptides in TFE. Different conformations corresponding to agonist and antagonist peptides were suggested by CD. Based on the pH-dependence of the **fluorescence** spectra of the peptides in a zwitterionic detergent, two titratable groups were identified (pKa = 6.3 and 8.5). The lower pKa is found in the agonist analogs but not in the .psi.[CH2S]-contg. antagonist.

=> d bib abs 4

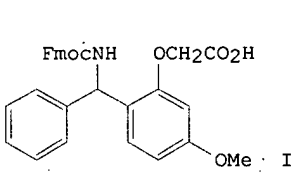
L51 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1990:199089 HCAPLUS
DN 112:199089
TI Solid-phase synthesis of bombesin by continuous flow procedure using Fmoc-amino acids
AU Scolaro, B.; Gozzini, L.; Rocchi, Raniero; Di Bello, C.
CS Biopolym. Res. Cent., Univ. Padova, Padua, 35131, Italy
SO Int. J. Pept. Protein Res. (1989), 34(5), 423-9
CODEN: IJPPC3; ISSN: 0367-8377
DT Journal
LA English
GI

pyroGlu-Gln-Arg-Leu-Gly-Asn-Gln-
Trp-Ala-Val-Gly-His-Leu-Met-NH_2 I

AB **Bombesin** (I) has been synthesized by the continuous flow solid-phase procedure on the derivatized Kieselguhr-supported polydimethylacrylamide resin. Preformed Fmoc (Fmoc = 9-**fluorenylmethoxycarbonyl**) amino acid sym. anhydrides (Met, Leu, and Arg) and Fmoc amino acid active esters were used for amine acylation. The 4-methoxy-2,3,6-trimethylbenzenesulfonyl and 2,2,5,7,8-pentamethylchroman-6-sulfonyl groups have been alternatively used for masking the side chain function of Arg-3. The progress of the synthesis was monitored by different anal. methods including quant. solid-phase Edman degrdn. Cleavage from the resin and simultaneous formation of the C-terminal **amide** function were achieved with a methanolic ammonia soln. yielding indistinguishable crude peptides which have been purified by HPLC and fully characterized. Preliminary pharmacol. expts. indicated that the activity of the synthetic peptides is similar to that previously measured for other synthetic **bombesins**. For comparison **bombesin** has also been prepd. by solid-phase synthesis on 4-methylbenhydrylamine resin using the Boc chem. The results of the two strategies are discussed and compared.

=> d bib abs 5

L51 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1990:199065 HCAPLUS
DN 112:199065
TI A comparison of acid-labile linkage agents for the synthesis of peptide C-terminal amides
AU Bernatowicz, Michael S.; Daniels, Scott B.; Koster, Hubert
CS MilliGen/Biosearch Div., Millipore, Burlington, MA, 01803, USA
SO Tetrahedron Lett. (1989), 30(35), 4645-8
CODEN: TELEAY; ISSN: 0040-4039
DT Journal
LA English
GI



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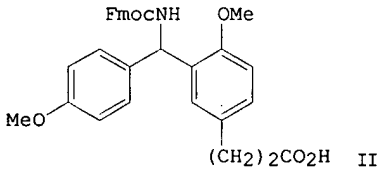

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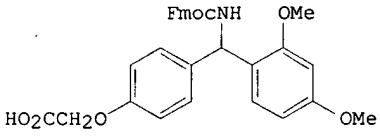

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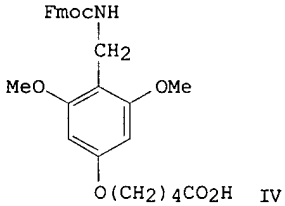

IV

AB Substituted benzhydrylamine and benzylamine linkage agents I-IV (Fmoc = 9-**fluorenylmethoxycarbonyl**) useful for the solid-phase peptide synthesis of C-terminal **amides** were evaluated for their relative lability toward CF3CO2H. The two most reactive linkage agents studied were compared in the synthesis of to different peptide **amides** (eledoisin and **neuromedin** U-25) by the Fmoc protecting group strategy.

=> d bib abs 6

L51 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2001 ACS
AN 1990:77970 HCAPLUS
DN 112:77970
TI Preparation of benzhydrylamine derivatives as reagents for solid-phase synthesis of polypeptide amides
IN Funakoshi, Susumu; Murayama, Eigoro
PA Chugai Pharmaceutical Co., Ltd., Japan
SO Eur. Pat. Appl., 11 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 331073	A2	19890906	EP 1989-103397	19890227
	EP 331073	A3	19901114		
	EP 331073	B1	19930728		
	R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
	JP 01221358	A2	19890904	JP 1988-46710	19880229
	US 4965405	A	19901023	US 1989-316167	19890227
	AT 92039	E	19930815	AT 1989-103397	19890227
	US 5051526	A	19910924	US 1990-527806	19900524
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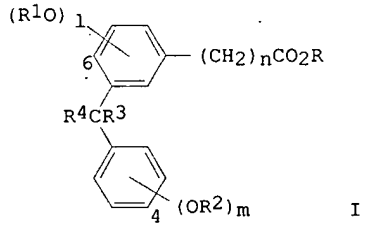
	EP 1989-103397		19890227		
	US 1989-316167		19890227		

OS MARPAT 112:77970
GI



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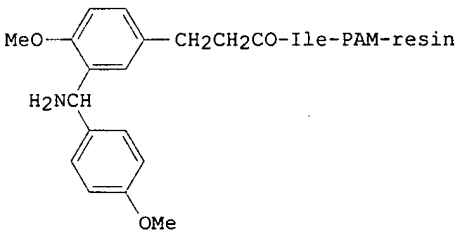

II

AB Benzhydrylamine derivs. (I; R = R4 = H; R1,R2 = C1-3 alkyl; l, m = 1, 2; n = 1-4; R3 = FMOCNH; FMOC = 9-**fluorenylmethyloxycarbonyl**), useful as reagents for modification of an aminomethylated polystyrene resin in the solid-phase synthesis of polypeptide **amides** using Fmoc protective group, are prepd. via intermediates I (R3 = H2N, R4 = H; R3R4 = O). Thus, Friedel-Crafts acylation of 4-MeOC6H4CH2CH2CO2Me with p-MeOC6H4COCl in PhNO2 in the presence of AlCl3 gave I [R = Me, (R1O)l = 6-MeO, (OR2)m = 4-OMe, R3R4 = O] which was sapond. with NaOH in aq. MeOH, oximated with H2NOH.HCl, and then reduced with Zn in AcOH to give I [R = R4 = H, (R1O)l = 6-MeO, (OR2)m = 4-OMe, R3 = NH2]. Acylation of the latter with O-(9-**fluorenylmethyloxycarbonyl**)-N-hydroxysuccinimide in DMF contg. Et3N gave I [R = R4 = H, (R1O)l = 6-MeO,

(OR2)m = 4-OMe, R3 = FMOCNH] which was coupled to H-Ile-PAM-polystyrene resin (PAM = phenylacetamidomethyl) in DMF in the presence of DCC and 1-hydroxybenzotriazole to give a modified resin (II). Tetragastrin, **neuromedin** B, and arginine-vasopressin were prepd. by the solid-phase method using II and FMOC protected amino acids.

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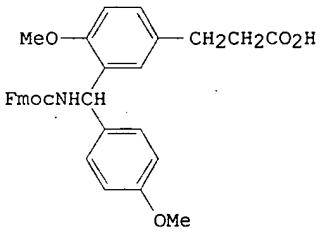

I

AB Dimethoxybenzhydrylamine deriv. I (Fmoc = 9-**fluorenylmethyloxycarbonyl**) was prepd. and used as a handle reagent for Fmoc-based solid-phase synthesis of peptide **amides** tetragastrin, **neuromedin** B, and [8-arginine]vasopressin. 1M Me3SiBr-PhSH (molar ratio 1:1) in CF3CO2H was recommended as a deprotecting reagent for releasing the peptide **amides** from the resin.